Journal of Horticultural Science & Biotechnology (2007) **82** (6) 960–966

Endogenous hormone concentrations and bud-break response to exogenous benzyl adenine in shoots of apple trees with two growth habits grown on three rootstocks

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SUMMARY
Scions from two siblings of a 'Goldspur Delicious' × 'Redspur Delicious' cross were budded to three rootstocks with different levels of vigour [M.9, M.7, and *Malus domestica* 'Antanovka' seedling] and planted in the field in 1997. The scions had two contrasting growth habits: one with narrow crotch angles, numerous short branches and an upright narrow (UN) canopy; and the other with wide crotch angles, few short branches, and a spreading round (SR) canopy. Shoot tips were collected at the time of bud-break in April 2004 and analysed for auxin (AUX), cytokinins (CK), and abscisic acid (ABA) to determine relationships between scion growth habit, size-controlling rootstock, and shoot tip hormone concentrations. Although not statistically different, the UN growth habit had numerically higher AUX, lower ABA, and equivalent CK levels as the SR growth habit. These differences resulted in statistically higher AUX:CK ratios (ACR). It is possible that the higher ACR contributed to the UN growth habit, which had more anti-gravitrophic shoot growth and appeared to have greater apical dominance than the SR growth habit. Either growth habit, grown on seedling rootstock, had nearly twice the ACR than on M.7 or M.9 rootstocks. The synthetic CK, 6-benzyl adenine (BA), was applied to 30 cm shoot explants of both growth habits in a greenhouse in March 2006. An 8.7 mM BA concentration stimulated bud-break in both growth habits, compared with controls, and bud-break was increased more in the UN than the SR growth habit. The results indicate that the ACR may be a factor regulating bud-break and the development of growth habit in apple scions, and that rootstock modified the hormone concentrations in shoot tips.

Apple tree (*Malus* × *domestica* Borkh.) growth habits are strongly affected by the phenology and ontogeny of the branches (Costes and Guédon, 2002). Branching can be modified intentionally by pruning and rootstock, but it may also be a less-calculated result of genetic selection when seeking superior fruit. Apple siblings can have diverse branching patterns and growth habits (Zagaja and Faust, 1983). A complex of signals, including hormones, may regulate branching (Beveridge *et al*., 2003). We reported previously on the interactive effects of apple growth habit and size-controlling rootstocks on current-year growth patterns and sylleptic branching (i.e., axillary bud growth, without a period of rest, from the current year's shoot; Tworkoski and Miller, 2007). In this experiment, we report the interactive effects of scion growth habit and rootstock on hormone concentrations in terminal buds of apple at the time of bud-break. We also report the effect of the application of exogenous cytokinins (CK) on bud-break of proleptic branches (i.e., axillary bud growth, following a period of rest; in this case from a previous year's shoot).

In apple shoots, sylleptic and proleptic branches develop in basitonic and acrotonic patterns, respectively (Costes and Guédon, 2002). These patterns suggest that branch development in apple may be correlatively regulated by processes such as apical dominance (i.e., distal meristem suppression of axillary bud-break) or apical control (i.e., distal meristem influence to reduce branch growth from an axillary bud). Branching patterns differed between siblings of a controlled cross that resulted in apple growth habits with upright, narrow (UN) or spreading, round (SR) canopies (Tworkoski and Miller, 2007). The distance from the terminal bud to the first sylleptic lateral branch was much greater in the UN than in the SR growth habit, suggesting that apical dominance may be greater in UN than SR. In addition, time to bud-break of axillary buds that could develop into proleptic branches differed between UN and SR trees, and rootstock had an interactive effect. The time to 50% bud-break was nearly three-times longer in UN trees on seedling rootstock than in UN trees on a more dwarfing rootstock, or in SR trees on any rootstock. Bud-break may be associated with competition from neighbouring buds, growth rate of the main axis, and/or hormone gradients (Costes and Guédon, 2002). A regulatory interaction occurs in pea (*Pisum sativum* L.) between shoot-produced auxin (AUX) and root-produced CKs (Bangerth *et al*., 2000). The transport, metabolism, and balance of these hormones can affect bud-break and canopy development.

Endogenous hormones have long been associated with patterns of bud-break and branch development. Sylleptic bud-break and branch development are regulated by AUX and CK concentrations (Bangerth *et al*., 2000; Bubán, 2000; Cline and Dong-Il, 2002; Thomas and Blakesley, 1987). Apically-produced AUX

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may suppress buds, and CKs produced by shoots and roots may promote bud growth (Cline, 1991). Reduced growth in dwarfed apple trees has been attributed to altered levels of endogenous hormones, including AUX, gibberellin, abscisic acid (ABA) and CK (Grochowska *et al*., 1984; Jaumien and Faust, 1984; Kamboj *et al*., 1999a; Looney *et al*., 1988; Steffens and Hedden, 1992). The AUX:CK ratio (ACR) has been found to affect bud-break in poplar, peach and other species (Cline and Dong-Il, 2002; Moncaleán *et al*., 2002; Sorce *et al*., 2002; Tworkoski *et al*., 2006). Tworkoski *et al*. (2006) found that the ACR was greater in peach trees with upright growth habits, and we hypothesised that similar differences might occur in apple trees. It is possible that the hormone ratio may be greater in certain growth habits; for example, the ACR may be higher in trees with more upright growth and greater apical dominance (i.e., the UN growth habit). Since roots are a source of CKs, it is possible that greater CK transport may originate from invigorating rootstocks to affect bud-break and branch development in apple.

Increased CK concentrations have been implicated in increased bud-break in apple (Cook and Bullstedt, 2001; Cutting *et al*., 1991). Cytokinin concentrations in xylem sap were greater in apple rootstocks that invigorated, rather than dwarfed, apple scion growth (Kamboj *et al*., 1999b). Exogenous applications of compounds with CK-like activity could stimulate bud-break and assist in the development of canopy structure in apple trees (Bubán, 2000; Kender *et al*., 1972; Steffens and Stutte, 1989). Since CKs could play an important role in growth form, and in the initiation of bud-break, we wanted to determine if bud-break could be enhanced in trees with longer times to bud-break (i.e., the UN growth habit). We were particularly interested in the effect of exogenous 6-benzyl adenine (BA) on trees with high ACRs. The hypothesis was that exogenous BA would stimulate bud-break more in trees with a high, rather than a low ACR.

The objectives of this experiment were: (1) to identify differences in AUX, ABA, and CK in the terminal buds of two apple scions with different growth habits grown on rootstocks with different capacities for size control; and (2) to determine the bud-break response to exogenous applications of CK to shoots from trees with different ACRs.

MATERIALS AND METHODS

The trees used in this experiment were described previously (Tworkoski and Miller, 2007). Briefly, the scion came from trees with different growth habits (UN or SR) that were seedlings from an F_2 generation of hybrids produced by sibcross selections from a 'Goldspur Delicious' × 'Redspur Delicious' progeny (Faust and Steffens, 1993; Zagaja and Faust, 1983). In 1996, scions were "T-budded" to rootstocks M.7 EMLA (semi-dwarfing); M.9 (dwarfing); and *M. domestica* 'Antanovka' (standard seedling). Ten trees of each scion-rootstock combination were planted into an orchard with 4.9 m × 4.9 m spacing on 21 October 1997. Other compound trees, not described here, were also planted in the orchard, but were not included in this experiment. The experimental layout was randomised with blocking based on location in the orchard. Trees were not pruned and were grown in 2 m-wide vegetation-free strips using pre-emergence herbicides in Spring and spot applications of paraquat (1,1'-dimethyl-4,4'-bipyridinium) or glyphosate [N-(phosphonomethyl) glycine], as needed. Insects and diseases were controlled using the local commercial spray recommendations (Pfeiffer, 1998). Tree growth (i.e., crotch angle, canopy height and width, 50% bud-break, and branching patterns) was measured in April 2003 and 2004, and those results have been reported elsewhere (Tworkoski and Miller, 2007).

Endogenous hormones

Twenty apical buds on each of nine UN and seven SR trees, on each rootstock, were sampled in April 2004, just as green shoots were emerging. Cytokinin concentrations in buds were elevated at this time, as CK levels in the xylem sap of apple increases up to the time of bud-break (Young, 1989). Buds from one tree were pooled to produce an experimental unit and were frozen quickly in liquid N_2 in the field, lyophilised, ground frozen, and stored at –80°C until analysed (Tworkoski *et al*., 2006; Wisniewski *et al*., 2006). The principal CKs were zeatin and zeatin riboside, which were added to provide the total cytokinin content. Treatment effects were evaluated by the Proc Mixed model with scion (i.e., growth habit) and rootstock being fixed, and the block being random effects. Means were separated by pdiff and Tukey's HSD test (SAS Version 8.02; SAS Institute, Cary, NC, USA).

Auxin and abscisic acid analysis: One g samples were extracted overnight at –20°C with 80% (v/v) methanol fortified with stable isotopes, phenyl-$^{13}C_6$ indole-3-acetic acid (IAA; Cambridge Isotope Laboratories, Andover, MA, USA) and 3',5',5',7',7',7'-D_6 ABA (Ken Nelson, National Research Council of Canada, Saskatoon, Saskatchewan, Canada) as internal standards. Butylhydroxytoluene (BHT; 16 mg l^{-1}) and ascorbic acid (10 mg l^{-1}) were added as anti-oxidants. Samples were centrifuged (2,000 × *g*, 18°C, 10 min), decanted, filtered, re-extracted, and the supernatants pooled. The supernatants were rotary flash evaporated (RFE), diluted with 6 ml 0.1 M K_2HPO_4 pH 8, and passed through a column of insoluble polyvinylpyrrolidone. The extracts were adjusted to pH 3 and separated on C18-columns (2 g high load capacity; Alltech, Lexington, KY, USA), that were pre-conditioned with 10 ml absolute methanol, then 20 ml pH 3 water, washed with 20 ml pH 3 water, eluted with 80% (v/v) methanol, and dried by RFE. The samples were then methylated with ethereal diazomethane (Cohen, 1984), evaporated, reconstituted in ethyl acetate, and quantified by gas chromatography-mass spectrometry (GC-MS), correcting for losses by using the internal standard.

Auxin and ABA were analysed with a gas chromatograph (5890 Series; Hewlett Packard, Palo Alto, CA, USA) equipped with a 30 m × 0.32 mm × 0.25 µm column (DB5; J&W Scientific, Folsom, CA, USA) and a mass selective detector (5971; Hewlett Packard). The injector and detector temperatures were 250ºC and 315ºC, respectively, and the oven temperature gradients were 60°C to 200°C at 5°C min^{-1}, 200°C to 300°C at 30°C min^{-1}, held at 300°C for 10 min, then from 300°C to

60°C at 50°C min^{-1}. Indole-3-acetic acid eluted at 24.9 min and was quantified by monitoring authentic IAA (m/z 130 and 189) and $^{13}C_6$-IAA (m/z 136, 195) with selective ion monitoring (100 ms dwell per ion; Cohen *et al*., 1986). ABA eluted at 29.6 min and was quantified by monitoring authentic ABA (m/z 190) and D_6-ABA (m/z 194) with selective ion monitoring (100 ms dwell per ion). The recovery averages of IAA and ABA were 30% and 45%, respectively.

Cytokinin analysis: Cytokinin extraction, purification, and quantitation followed the procedure of Moritz and Sundberg (1996) with modification. One g dry weight (DW) of tissue was extracted overnight in 20 ml extraction solution [80% (v/v) methanol, 20% (v/v) 0.02 M potassium phosphate buffer, plus 16 mg l^{-1} BHT and 10 mg l^{-1} ascorbic acid] at –20°C. The extraction solution was "spiked" with stable isotopes of each cytokinin: [^{15}N] *trans*-zeatin ([^{15}N]-tZ) and [D_5] *trans*-zeatin riboside ([D_5]-tZR); Olchemim, Olomouc, Czech Republic). The solution was centrifuged (2,000 × *g*, 18°C, 10 min), decanted, re-extracted with 10 ml extraction solution, centrifuged and the supernatants pooled. The supernatants were evaporated to an aqueous residue by RFE, treated with phosphatase (0.04 Units ml^{-1} acid phosphatase; EC 3.1.3.2; Sigma Chemical Co., St. Louis, MO, USA) and incubated for 30 min at 37°C. The sample was then loaded onto an anion exchange column (10 g SAX; Varian, Palo Alto, CA, USA) linked to a C18-column (2 g high load capacity; Alltech) that had been pre-conditioned with 15 ml absolute methanol followed by 20 ml 0.02 M potassium phosphate buffer, pH 7.2. After loading the sample, the column was washed with 40 ml 0.02 M potassium phosphate buffer, disconnected and the C18-column washed with 15 ml water. Cytokinins were eluted from the C18-column with 10 ml 80% (v/v) methanol. The eluant was evaporated to near dryness by RFE, re-suspended in 1 ml 0.01 M ammonium acetate buffer pH 3, loaded onto a strong cation exchange column (0.5 g SCX; Varian) that had been pre-conditioned with 10 ml 0.01 M ammonium acetate buffer pH 3. The column was washed with 10 ml 0.01 M ammonium acetate buffer pH 3, and the cytokinins were eluted with 2 M ammonia in methanol and evaporated to dryness by RFE. The sample was reconstituted and assayed by high performance liquid chromatography-mass spectrometry (HPLC-MS).

The HPLC method used to separate cytokinins was a modification of the method used by Suttle (1998). A 1 µl sample was injected into an HPLC equipped with a binary pump (G1321A, 1100 Series; Agilent, Santa Clara, CA, USA), a C18 guard column (Bio-Sil HL90-5; Bio-Rad, Hercules, CA, USA), a C18-column (0.8 cm × 10 cm NovaPak; Waters Associates, Milford, MA, USA), and a mass spectrometer with electrospray ionisation (G1956B; Agilent). The solvent flow rate was 0.5 ml min^{-1} with solvent A being 1% (v/v) acetic acid and solvent B being acetonitrile. Solvent delivery was 5% (v/v) B in solvent A for 10 min, a linear gradient to 30% B from 10 – 35 min, 30% (v/v) B from 35 – 40 min, a linear gradient to 100% (v/v) B from 40 – 45 min, 100% (v/v) B from 45 – 58 min, and a linear gradient to 5% (v/v) B in solvent A from 58 – 63 min.

Cytokinin quantitation was based on a modification of the method used by Novak *et al*. (2003). The electrospray conditions were: capillary voltage +3.0 kV, cone voltage +100 V, desolvation temperature 350ºC, nitrogen drying gas 12.0 l min^{-1}, and a nebuliser pressure of 241 kPa. Quantitation was done using selected ion monitoring (SIM) of quasi-molecular ions $(M + H)^+$ of extracted and internal standard stable isotopes of each cytokinin: tZ (220) and [^{15}N]-tZ (221) at 27.2 min, and tZR (352) and [D_5]-tZR (357) at 29.5 min. Cytokinin recovery averaged between 30 – 40%.

Cytokinin quantitation included a correction for losses due to the isotope dilution-relative response method (EPA Method 1625). Isotope ratios were determined for the quasi-molecular ions noted above from the non-isotope standards alone, the stable isotope standards alone, and mixtures of non-isotope and stable isotope standards. The following mixtures of non-isotope cytokinin and isotope cytokinin were prepared (ng $µl^{-1}$): 25:0, 0:25, 25:10, 25:5, 25:1, and 25:0.5, injected for each cytokinin, and the LC-MS run as a standard relative response *vs.* concentration curve:

$$\text{Relative response} = (Ry - Rm)(Rx + 1) / (Rm - Rx)(Ry + 1).$$

where Rx = the quasi-molecular ion ratio for the non-isotope cytokinin, Ry = the quasi-molecular ion ratio for the isotope cytokinin, and Rm = the quasi-molecular ion ratio for the mixtures of non-isotope cytokinin and isotope cytokinin prepared as described above.

Exogenous BA

In March 2006, ten distal shoots per tree, each 30 cm-long, were collected from the UN and SR trees described above, and were treated with the synthetic cytokinin, benzyl adenine (BA). Benzyl adenine (BA formulated in MaxCel; Valent BioSciences, Libertyville, IL, USA) was applied at 0, 0.87, 8.7, and 87 mM by brushing an aqueous solution onto each branch after it was cut and placed in the greenhouse. Branches were maintained at 26°C in the greenhouse under a 12 h photoperiod with supplemental lighting, and the cut ends were kept submerged in water. Beginning on the day that the stems were placed in the greenhouse (t = 0; i.e., no buds had yet broken), the number of growing buds were counted every second day. The number of growing buds (determined if 1 mm of green tip emerged) on each branch was counted until bud-break had ceased for four consecutive days.

Treatment effects were analysed as described above. To account for differences in the total number of buds 30 cm^{-1} shoot, bud-break for each BA treatment was calculated as a percentage of the controls (0 mM BA) within each tree (i.e., experimental unit) as:

$$\text{Number of buds broken (\% of control)} = [\text{No. buds broken for a specified BA treatment of one tree} / \text{No. buds broken for 0 mM BA treatment of that same tree}] \times 100$$

The effects of BA on bud-break were determined as a randomised design that included the two growth habits (UN and SR), three rootstocks (M.9, M.7, *M.* ×

TABLE I
Main effects of growth habit and rootstock on auxin (AUX), cytokinin (CK), abscisic acid (ABA), and auxin:cytokinin ratios (ACR) in apical buds of 8-year-old trees grown in the field at bud-break in April 2004

Effect*		AUX (pmol g^{-1} DW)	CK (pmol g^{-1} DW)	ABA (pmol g^{-1} DW)	ACR (w/w)
Growth habit (GH)†	UN	530 a	129 a	742 a	4.1 a
	SR	370 a	122 a	1027 a	3.0 b
Rootstock (R)‡	M.9	479 a	142 a	910 a	3.3 b
	M.7	347 a	109 a	1017 a	3.1 b
	Seedling	619 a	102 a	617 a	6.1 a
Significance (*P*)					
GH		0.10	0.95	0.38	0.01
R		0.12	0.15	0.30	0.05
GH × R		0.39	0.81	0.16	0.35

*Treatment effects were determined by the Proc Mixed model, with scion and rootstock being fixed and block being the random effects. Within each main effect, means followed by the same lower-case letter do not differ at $P = 0.05$ based on Tukey's HSD test.
†UN and SR were growth habits with upright narrow, or spreading round canopies, respectively.
‡M.9, M.7, and seedling were dwarfing, semi-dwarfing, and invigorating rootstocks, respectively.

domestica 'Antanovka'), four rates of BA (0, 0.87, 8.7, or 87 mM), and three replications (one tree was an experimental unit) with ten sub-samples (30 cm shoot tips) per experimental unit. Contrast analysis was performed on log-transformed data to evaluate the function that best described bud-break response to BA rate.

RESULTS

Endogenous hormones

Growth habit × rootstock interactions were not found. The main effects of growth habit and rootstock are presented in Table I. The ACR was significantly affected by growth habit, and by rootstock ($P = 0.01$ and 0.05, respectively), but individual hormones generally were not. The ACRs were higher in the tips of apple shoots with the UN, compared to the SR growth habit (Table I). This effect was mostly associated with greater AUX concentrations ($P = 0.10$). Cytokinin and ABA concentrations did not differ between growth habits (Table I). Among the rootstocks, ABA concentrations did not differ, although ABA concentrations tended to be higher in the shoot tips of trees grown on M.9 and M.7 than on seedling rootstocks. Auxin concentrations tended to be lowest in scions on M.7, and cytokinin concentrations highest in scions on M.9 rootstocks (Table I). However, the ACRs were the same in scions on both M.7 and M.9 rootstocks, and were significantly lower than in shoot tips from scions grown on seedling rootstock.

Exogenous BA

The number of buds that broke on 30 cm-long distal shoots was consistently greater in the SR than in the UN growth habit, and on M.9 than on seedling rootstock after 4 d in the greenhouse (Table II). Application of 8.7 mM BA increased the number of buds that broke. The 87 mM treatment killed nearly all the buds (Figure 1). In general, growth habit × rootstock interactions occurred over 8 d in the greenhouse (Table II), and resulted from the M.9 and M.7 rootstocks

TABLE II
Rootstock, scion, and 6-benzyl adenine (BA) treatment effects on bud-break up to 14 d after BA treatment of 30-cm apple shoots placed in a greenhouse on March 2006

		Days in the greenhouse					
Main effect*		4	6	8	10	12	14
		(Number of buds broken)					
Growth habit (GH)†	UN	8 b	30 b	62 b	68 b	72 b	72 b
	SR	52 a	77 a	82 a	82 a	85 a	87 a
Rootstock (R)‡	M.9	36 a	42 a	70 a	79 a	82 a	82 a
	M.7	30 ab	45 a	71 a	78 a	82 a	83 a
	Seedling	26 b	42 a	68 a	70 a	72 a	73 a
BA (mM)††	0	27	48	64	68	71	71
	0.87	30	50	64	66	67	67
	8.7	35	64	88	92	98	100
Response to BA**		L	L	L	L/Q	L/Q	L/Q
Significance (*P*)							
GH		0.01	0.01	0.01	0.02	0.03	0.01
R		0.01	0.40	0.33	0.40	0.29	0.30
BA		0.05	0.01	0.01	0.01	0.01	0.01
R × BA		0.36	0.58	0.97	0.89	0.96	0.97
GH × BA		0.36	0.85	0.46	0.34	0.59	0.75
GH × R		0.01	0.01	0.02	0.28	0.48	0.56
GH × R × BA		0.24	0.44	0.81	0.89	0.92	0.92

*Treatment effects were determined by the Proc Mixed model, with scion and rootstock being fixed and block being random effects. Within each main effect and day, means followed by the same lower-case letter do not differ at $P = 0.05$ based on Tukey's HSD test.
†UN and SR were growth habits with upright narrow, or spreading round canopies, respectively.
‡M.9, M.7, and seedling were dwarfing, semi-dwarfing, and invigorating rootstocks, respectively.
††BA, exogenously applied benzyl adenine.
**L and Q represent significant linear and quadratic effects, respectively, at $P < 0.05$ based on contrast analysis.

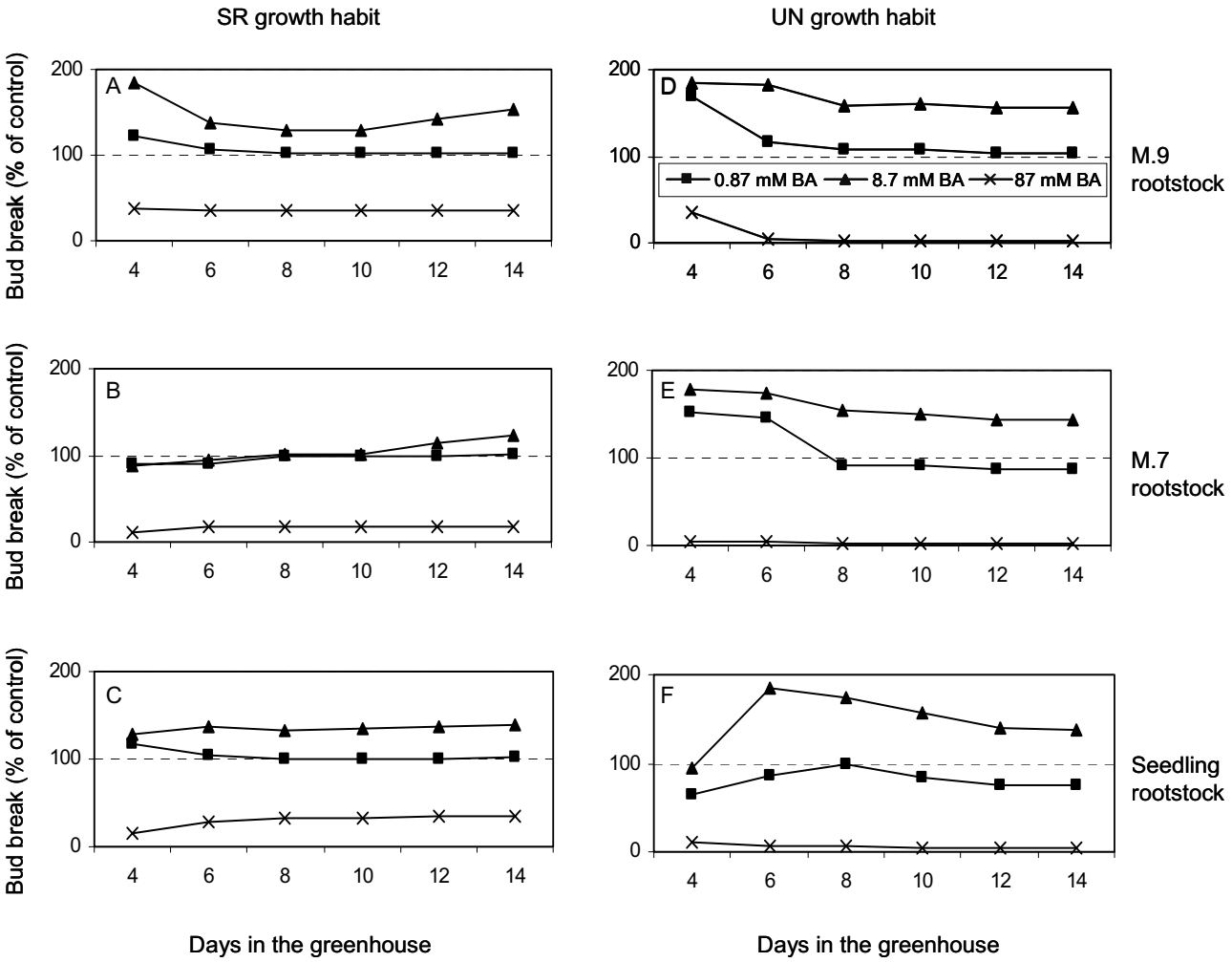

FIG. 1
Bud-break in 30-cm shoot tip explants of Spreading Round (SR; Panels A-C) and Upright Narrow (UN; Panels D-F) growth habits of apple on rootstocks M.9 (Panels A and D), M.7 (Panels B and E), and seedling (Panels C and F), in a greenhouse, up to 14 d after treatment with 0.87, 8.7, or 87 mM 6-benzyl adenine (BA). Bud-break is expressed as the percentage of control (0 mM BA). All 87 mM and none of the 0.87 mM BA treatments differed from the control by LSD (P = 0.05). All 8.7 mM BA treatments, except SR on M.7 at 8 d and 10 d in the greenhouse, differed from the control.

stimulating a greater total bud-break in the SR than in the UN growth habit (data not shown). Benzyl adenine interacted with growth habit to affect the percentage bud-break relative to the control (Figure 1). Benzyl adenine increased bud-break more in UN than in SR, on all dates after transfer to the greenhouse (Figure 1). In particular, BA enhanced bud-break in UN compared with SR on M.7 rootstock.

DISCUSSION

Endogenous hormones

The ACRs were higher, and auxin concentrations were numerically greater, in apple shoot tips with the UN compared to the SR growth habit (Table I). Auxins produced and translocated from the apical meristem can inhibit subtending laterals and contribute to apical dominance in numerous species, including apple (Bangerth, 1989; Faust *et al.*, 1997). There were more internodes and greater distances between the terminal bud and the first sylleptic branch in UN than in SR, suggesting stronger apical dominance in the UN growth habit (Tworkoski and Miller, 2007). High CK concentrations in shoots can reduce the influence of apical meristems on inhibiting the growth of axillary buds. High CK concentrations early in the season induced bud-break of proleptic shoots, and high CK concentrations in the distal portion of 1-year-old shoots promoted strong acrotony in apple (Cook *et al.*, 2001; Cutting *et al.*, 1991). In the current study, CK concentrations in terminal buds at the beginning of the growing season did not differ between growth habits (Table I). However, the ACR was higher in UN than in SR trees. Auxin-cytokinin interactions may affect a number of processes that regulate bud growth, including apical dominance (Bangerth, 1994). Our data suggest that AUX concentrations influenced growth habit in apple, possibly by interacting with CKs; but that CKs did not differ between growth habits.

In the current experiments, the ACR was the same in shoot tips of scions on both M.7 and M.9 rootstocks, and this ACR was significantly lower than in shoot tips from scions grown on seedling rootstock (Table I). The higher ACR associated with seedling rootstock may reflect the capacity of seedling rootstock to invigorate terminal bud growth, possibly through increased availability of edaphic resources. Invigorating rootstocks can have greater internal xylem conductance than dwarfing

rootstocks (Atkinson *et al*., 2003). More vigorous rootstocks promoted apical dominance in UN, but not in SR, compared with less vigorous rootstocks (Tworkoski and Miller, 2007). As with growth habit, increased apical dominance with rootstock appeared to be associated with an increased ACR.

Rootstocks may induce size-controlling effects on scions *via* several mechanisms, including translocation of hormones. Kamboj *et al*. (1997) found higher CK concentrations in the sap from invigorating MM.106, than from dwarfing M.9 rootstocks. In the current study, shoot tips on more invigorating seedling rootstock had numerically lower CK levels than the most dwarfing rootstock, M.9 (Table I). This apparent difference may, in part, be due to the plant part measured. Xylem exudate likely reflects hormones originating from the roots. In the buds sampled in this experiment, the shoot may have supplemented root-produced CKs. Shoots and leaves have been demonstrated to be important sources of CK in apple trees (Cook *et al*., 2001; Greene, 1975). It is possible that M.9 rootstock may have induced CK synthesis, or release from sequestration, which may contribute to the earlier bud-break measured in scion grown on M.9 (Table II).

Concentrations of ABA in shoots on M.7 and M.9 rootstocks were numerically greater than, but not significantly different from seedling rootstock (Table I). Abscisic acid inhibited bud growth in apple when injected into the xylem stream (Sterrett and Hipkins, 1980). This inhibition could be overcome by follow-up injections of BA. Abscisic acid was also higher in shoot bark of dwarfing than invigorating rootstocks (Kamboj *et al*., 1999a). The higher ABA levels may have increased phloem differentiation, and the resulting high bark-to-wood ratio could have reduced xylem conductivity in dwarfing rootstocks. In the current study, the higher ABA in the shoot tips of trees grown on dwarfing rootstock may be attributed to higher ABA production in the rootstock, since ABA can move acropetally from roots *via* xylem (Davies *et al*., 2005).

Exogenous BA

Time to 50% bud-break of proleptic branches averaged 2.6 d for SR trees, regardless of rootstock, and 3.7 d for UN trees on M.9 and M.7, but 8.8 d for UN trees on seedling rootstock (Figure 2; Tworkoski and Miller, 2007). As reported above, the ACR in shoot tips was 6.1 on seedling rootstock and approx. 3.0 on M.9 and M.7 rootstocks (Table I). The ACR also was higher in scions with the UN (4.1) than with the SR (3) growth habit. We hypothesise that the longer time for bud-break in UN on seedling rootstock may have been associated with a higher ACR in UN than in SR and seedling, rather than M.7 and M.9 rootstocks. To test this hypothesis, BA was applied to alter the ACR. We anticipated greater stimulation of bud-break in UN scions on seedling rootstock.

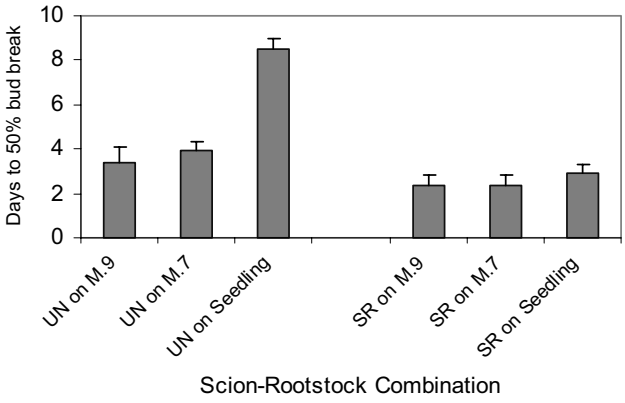

FIG. 2
Time to 50% bud-break in shoots in two apple growth habits on three rootstocks. Individual scion-rootstock combinations are presented due to significant scion-rootstock interactions (derived from Tworkoski and Miller, 2007). Bars represent one standard error (n = 3). Scion growth habits were Spreading Round (SR) and Upright Narrow (UN) on three rootstocks (M.9, M.7, and *M.* × *domestica* 'Antanovka' seedling).

Benzyl adenine increased bud-break in UN more than in SR, on all dates after placement in the greenhouse (Figure 1; Table II). When expressed as a percentage of control, BA treatments interacted with growth habit. These results confirm previous findings that exogenous applications of CK can increase bud-break in apple trees (Steffens and Stutte, 1989; Sterrett and Hipkins, 1980). Our results also support the hypothesis that bud-break was more responsive to BA in trees with higher (UN) than lower (SR) ACRs. Exogenous applications of compounds with CK-like activity can stimulate bud-break and assist in the development of canopy structure in apple trees (Bubán, 2000). Apple cultivars with different growth habits have been found to respond differently to exogenous cytokinins (Miller and Eldridge, 1986). The results from the current experiment can explain, in part, the branching response to exogenous BA treatments that can vary with growth habit and rootstock.

Size-controlling rootstocks probably have a number of primary and secondary effects on chemical, hydraulic, and nutritional messages. Results from the current experiments indicate that rootstocks can influence hormone concentrations, but the results were not as simple as dwarfing rootstock producing high or low quantities of ABA, AUX or CK. In the apple growth habits in this experiment, the ACR appeared to be lower in the dwarfing than in the invigorating rootstock. Improved knowledge of how such signals interact with each other, and with exogenous regulators, can help efforts to grow fruit trees to the specifications needed for efficient orchard management.

We would like to thank Tom Millay for technical assistance. We also thank D. M. Glenn and F. Takeda for reviews of an earlier version of this manuscript.

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